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Biochemical and hematologic profiles of 1000 submariners

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Tappan, D. V., M. J. Jacey, E. Heyder, and C. A. Harvey. 1979. Biochemical and hematologic profiles of 1000 submariners. Undersea Biomed. Res. Sub. Suppl.: S191–S199.—Control biochemical and hematologic data were gathered for 1017 healthy submariners who ranged in age from 19.5 to 43.5 years. Means, standard deviation, and frequency distribution are presented for 24 whole blood and serum variables and, where appropriate, for 11 urinary variables. After statistical separation of the effects of aging and length of submarine service, it has been determined that the following correlations were significant in this sample: neutrophil and leucocyte levels, serum cholesterol, and both fasting and postprandial glucose correlated positively with age; serum alkaline phosphatase levels and age correlated negatively. Age-corrected positive correlations were demonstrable between length of submarine service and both serum cholesterol content and alkaline phosphatase activity; after a loading test, glucose levels showed a negative relationship to length of submarine service. Split-sample correlation analyses verified these significant correlations, with the exception of the apparent rise in alkaline phosphatase activity with increasing length of submarine duty.

submarine health
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This Laboratory has been engaged for approximately the last three years in a study designed to evaluate any biochemical effects of submarine service on the health of men serving in this branch of the Navy. Since data from a sizable population of healthy subjects are accumulating, it is probable that such information will be a useful reference for other populations or demographic subgroups. The information gathered from this group of 1017 men will serve as reference data for continued investigations of submariners with varying amounts of total submergence time and of crews participating in active patrols.

A preliminary description of biochemical data obtained from approximately the first half of the subjects examined in this series of studies has been presented (Tappan, Jacey, Heyder, and Tansey 1975). The categories of data discussed in that paper have been extended in the present study to include the results of hematologic evaluations and semi-quantitative urine analyses as well as the previously described serum chemistry parameters.

MATERIALS AND METHODS

The subjects of the continuing study discussed in this report are members of the submarine service of the U.S. Navy who have training and experience appropriate to their designation as qualified submariners. The age of each subject was calculated according to the date on which he underwent the comprehensive test series called the Longitudinal Health Examination. Complete descriptions of the series have been given by Sawyer with Baker (1972) and by Tansey (1974). Length of submarine service was taken to be the number of years to the nearest year since qualification for submarine duty.

For purposes of standardization of biochemical data, the subjects were instructed to fast overnight before reporting at 7 a.m. for the examination series. The blood samples used for the present analyses consisted of part of a total of 40 ml of blood drawn from an antecubital vein. For the serum analyses, 7.5 ml of blood was drawn into a vacuum tube that did not contain anticoagulant and was allowed to clot thoroughly. A second specimen was used for the preparation of serum for frozen storage to meet future analytical requirements, while a third sample was collected into an oxalated tube for hematologic analysis. An additional blood sample was withdrawn two hours after the oral administration of 100 g glucose per subject for a glucose tolerance test. Subjects were recalled for re-evaluation if abnormal glucose tolerance was observed.

Except for the glucose determinations, the serum chemistry data indicated in Table 1 were obtained by an automated analytical method, SMA 12/60 (Technicon Corp.). Glucose measurements were made by the Diagnostest procedure (Dow Chemical Co.). Hemoglobin, hematocrit and total white cell counts were obtained using a Model S analyzer (Coulter Corp.), with the leucocyte differential counts made manually by currently accepted hematology procedures (Miale 1972). Urine samples were analyzed by the Labstix Test (Ames Laboratories), evaluated for specific gravity by hydrometry, and examined microscopically for sediment after centrifugation (Davidson and Wells 1963).

RESULTS

Biochemical characteristics of the submariners examined are shown in Tables 1 and 2. Table 1 shows a summary of frequency distributions for each group of biochemical and hematologic data, as well as for age and length of submarine service. In the first three data columns of the table, high and low values for the frequency distributions are presented along with the size of the intervals used for grouping the data between the extremes. In the case of the age distribution, for example, the data should be interpreted to indicate that 9 subjects were less than or equal to 19.5 years of age, i.e., <= Val (value) 1, and 7 subjects were older than 43.5 years, value 11. Steps between are 2-year intervals: 122 subjects in the interval >19.5 to 21.5 years, 196 from 21.5 to 23.5 years, and so on. Data for the other parameters are interpreted similarly. Means and standard deviations for each of the variables are also presented in this table. Median and modal values and skewness and kurtosis data describing the distribution curves may be obtained from the authors.

In a manner similar to that of Table 1, Table 2 summarizes the data distributions for the urinalysis examinations and provides mean and standard deviation values for the specific gravity analyses. Table 3 presents correlation values between biochemical data and years spent in submarine service (SUBM) or age of the subjects. Since a strong relationship would be expected to exist between the effect of age and the length of service for any parameters that are age-dependent, correlations are presented against both age and service time, with correc-

TABLE 1

FREQUENCY DISTRIBUTIONS, MEANS, AND STANDARD DEVIATIONS FOR AGE, SUBMARINE SERVICE, AND WHOLE BLOOD AND SERUM VARIABLES

	VAL 1†	Val II	INTRVL	<=1	1–2	2–3	3–4	4–5	5–6	6–7	7–8	8–9	9–10	10–11	>11	Mean	SD
AGE	19.5	43.5	2.0	9	122	196	167	85	109	102	107	72	29	10	7	28.44	5.89
SUBM*	1.0	21.0	2.0	191	266	120	85	51	111	71	40	23	11	5	4	5.87	4.87
HCT	36.0	56.0	2.0	4	0	13	68	141	288	264	142	54	15	7	10	46.57	3.10
HGBN	11.0	21.0	1.0	1	1	5	23	152	399	309	86	19	5	3	3	15.93	1.45
WBCS	25.0	125.0	10.0	1	3	41	148	239	243	162	82	39	19	18	10	70.43	17.79
NEUT	28.0	78.0	5.0	3	9	17	44	123	217	242	183	121	34	20	2	55.48	8.56
LYMP	20.0	60.0	4.0	11	18	59	113	171	190	192	117	73	40	21	9	39.63	8.34
MONO	0.0	10.0	1.0	148	188	176	161	124	81	70	30	17	10	7	4	2.85	2.27
BNDS	0.0	10.0	1.0	948	24	6	3	0	0	0	0	0	0	0	1	0.07	1.12
EOSN	0.0	10.0	1.0	296	272	166	115	76	30	23	14	12	4	2	6	1.85	2.15
BASO	0.0	10.0	1.0	920	82	12	1	1	0	0	0	0	0	0	0	0.11	0.37
CA	8.0	11.0	0.3	7	0	9	21	116	218	264	218	120	28	7	1	9.69	0.48
PHOS	2.5	5.5	0.3	6	21	41	88	154	240	201	164	68	22	4	5	3.99	0.58
BUN	8.0	28.0	2.0	5	43	98	203	243	201	122	52	32	4	4	7	16.25	5.42
UA	3.2	9.2	0.6	2	3	17	83	168	252	206	144	84	31	15	9	6.43	2.82
CHOL	120.0	320.0	20.0	5	16	57	135	239	236	159	92	47	12	9	7	207.28	37.97
TPR	5.7	8.7	0.3	2	4	4	21	102	261	276	212	95	29	3	1	7.38	0.42
ALBN	3.6	5.6	0.2	3	12	57	148	255	150	198	130	43	9	4	4	4.49	0.34
GLBN	1.2	4.2	0.3	3	2	5	29	94	202	321	215	110	24	5	1	2.90	0.43
A/G	0.8	2.8	0.2	5	1	67	238	307	196	96	55	31	7	4	6	1.62	1.40
TBIL	0.2	2.2	0.2	18	204	399	226	86	36	17	4	9	2	5	8	0.66	0.42
ALKP	20.0	120.0	10.0	3	9	55	166	212	216	164	90	46	25	12	12	66.11	31.11
LDH	50.0	300.0	25.0	2	13	69	139	270	231	177	74	24	7	2	5	154.38	50.77
SGOT	10.0	110.0	10.0	26	146	143	282	240	93	43	19	8	3	1	6	38.15	17.97
GLUC	50.0	150.0	10.0	3	9	39	153	342	307	99	32	15	6	4	5	90.47	14.60
2HPP	50.0	150.0	10.0	16	35	95	153	229	190	115	80	41	22	10	16	91.69	22.71

*SUBM = years of submarine service; HCT = hematocrit, %; HGBN = hemoglobin, g/dl; WBC = leucocytes, $\# \times 10^{-2}/mm^3$; NEUT = neutrophils; BND = band neutrophils; LYMP = lymphocytes; EOSN = eosinophils; MONO = monocytes; BASO = basophils - all are % WBC; CA = total serum calcium, mg/dl; PHOS = phosphate, mg/dl; BUN = urea nitrogen, mg/dl; UA = uric acid, mg/dl; CHOL = cholesterol, mg/dl; TPR = total protein, g/dl; ALBN = albumin, g/dl; GLBN = globulin, g/dl; A/G = albumin/globulin ratio; TBIL = total bilirubin, mg/dl; ALKP = alkaline phosphatase, mu/ml; LDH = lactic dehydrogenase, mu/ml; SGOT = glutamic-oxalacetic transaminase, mu/ml; GLUC = fasting glucose, mg/dl; 2HPP = 2-h postprandial glucose, mg/dl. †See text for explanation of column values.

TABLE 2

FREQUENCY DISTRIBUTION FOR QUALITATIVELY AND SEMI-QUANTITATIVELY MEASURED URINE COMPONENTS

Variable	0	1	2	3	4
AMOR*	815	201			
CRST*	1002	14			
HYLN*	1008	8			
CWBC*	1002	12			
CRBC*	1012	1			
MWBC	650	266	70	20	10
MRBC	1005	1	2	3	5
OCBL†	1011	2	1	0	
SUGR†	1009	2	1	1	3
ALBN†	997	2	1	0	2
SPGR	21	96	391	425	83

*0 = Negative, 1 = positive; †observations graded qualitatively, 0–3 or 0–4; AMOR = amorphous sediment; CRST = crystalline sediment; HYLN = hyalin casts; CWBC = WBC casts; CRBC = RBC casts; MWBC = microscopic WBC's, highest No. in 1 high-power field (distribution groups: 0, 2–5, 6–21, 22–51, 70–99); MRBC = microscopic RBC's (distribution groups: 0, 1, 2, 3, 4–15); OCBL = occult blood; SUGR = positive glucose test; ALBN = positive albumin; SPGR = specific gravity (grouping: 1.002–1.012; 1.013–1.018; 1.019–1.024; 1.025–1.030; 1.031–1.036). Mean SPGR = 1.024 ± .005 (SD).

TABLE 3

CORRELATION OF BLOOD, SERUM, AND URINE VARIABLES WITH AGE OF SUBJECTS AND YEARS OF SUBMARINE SERVICE

1	2	3	R12	R12.3
HCT	SUBM	AGE	−0.0359	−0.0048
HCT	AGE	SUBM	−0.0404	−0.0192
HGBN	SUBM	AGE	−0.0505	−0.0033
HGBN	AGE	SUBM	−0.0593	−0.0313
WBCS	SUBM	AGE	0.0794*	−0.0077
WBCS	AGE	SUBM	0.1022*	0.0650*
NEUT	SUBM	AGE	0.0646*	−0.0251
NEUT	AGE	SUBM	0.0962*	0.0757*
LYMP	SUBM	AGE	−0.0702*	−0.0074
LYMP	AGE	SUBM	−0.0804*	−0.0400
SPGR	SUBM	AGE	−0.0540	−0.0028
SPGR	AGE	SUBM	−0.0639*	−0.0343
CA	SUBM	AGE	−0.0496	−0.0526
CA	AGE	SUBM	−0.0238	0.0295
PHOS	SUBM	AGE	−0.0086	−0.0462
PHOS	AGE	SUBM	0.0217	0.0503
BUN	SUBM	AGE	0.0576	0.0475
BUN	AGE	SUBM	0.0371	−0.0178
UA	SUBM	AGE	0.0498	−0.0065

TABLE 3—Continued

1	2	3	R12	R12.3
UA	AGE	SUBM	0.0653*	0.0428
CHOL	SUBM	AGE	0.2959*	0.0648*
CHOL	AGE	SUBM	0.3179*	0.1376*
TPR	SUBM	AGE	0.0092	−0.0085
TPR	AGE	SUBM	0.0172	0.0169
ALBN	SUBM	AGE	−0.0101	−0.0438
ALBN	AGE	SUBM	0.0182	0.0463
GLBN	SUBM	AGE	0.0205	−0.0141
GLBN	AGE	SUBM	0.0348	0.0314
A/G	SUBM	AGE	−0.0124	0.0004
A/G	AGE	SUBM	−0.0154	−0.0091
TBIL	SUBM	AGE	0.0015	−0.0495
TBIL	AGE	SUBM	0.0363	0.0613
ALKP	SUBM	AGE	0.0139	0.0712*
ALKP	AGE	SUBM	−0.0327	−0.0771*
LDH	SUBM	AGE	−0.0293	−0.0479
LDH	AGE	SUBM	−0.0023	0.0380
SGOT	SUBM	AGE	−0.0182	−0.0285
SGOT	AGE	SUBM	−0.0023	0.0221
GLUC	SUBM	AGE	0.0333	−0.0361
GLUC	AGE	SUBM	0.0657*	0.0671*
2HPP	SUBM	AGE	0.0340	−0.0698*
2HPP	AGE	SUBM	0.0900*	0.1086*

R12 = Correlation of Col 1 vs. Col 2 variables; R12.3 = correlation of Col 1 vs. Col 2 variables corrected for effect of Col 3 variable. *Correlation is statistically significant, $P < 0.05$. HCT = Hematocrit; HGBN = hemoglobin; WBCs = leucocytes; NEUT = neutrophils; LYMP = lymphocytes; SPGR = urine specific gravity; CA = serum calcium; PHOS = phosphate; BUN = urea nitrogen; UA = uric acid; CHOL = cholesterol; TPR = total protein; ALBN = albumin; GLBN = globulin, g/dl; A/G = albumin/globulin ratio; TBIL = total bilirubin; ALKP = alkaline phosphate; LDH = lactic dehydrogenase; SGOT = glutamic-oxalacetic transaminase; GLUC = fasting glucose; 2HPP = 2-h postprandial glucose.

tions made for the other component. Corrections were made by the method of partial correlation described by Snedecor and Cochran (1967).

Though several of the biochemical and hematologic parameters apparently correlate significantly with age and/or length of submarine service (Column R12, Table 3), a considerably altered picture emerges (Column R12.3, Table 3) when the effects of age and submarine service are separated. Total number of white blood cells and neutrophils, fasting and 2-h postprandial glucose, and cholesterol may be seen to correlate positively and significantly with age after correlation effects attributed to length of submarine service are removed. Alkaline phosphatase levels, on the other hand, correlate negatively with age after removal of the effect of time in the service. In addition, significant age-corrected positive correlations between length of submarine service and cholesterol values, as well as alkaline phosphatase concentrations, may be seen. Two-hour postprandial glucose levels show a significant negative relationship to length of submarine service.

DISCUSSION

Since this sample is a highly selected subgroup of a larger, healthy population, i.e., active-duty Naval personnel, it is not surprising that the data fall within ranges that are considered normal for the population. This general conclusion is documented and discussed in detail in a previous report of the serum chemistry data obtained for an initial phase of this study (Tappan et al. 1975). As expected, the group means and distributions for the earlier series are in close agreement with the final data tabulated here. Since the previous report discussed the data of individual parameters in some detail with respect to the results of other investigators, it does not seem appropriate or necessary to address separately the serum, blood, or urinalysis data at this time. Only those of the present data related to the aging process or to unique occupational factors will be discussed below.

An examination of column R12 of Table 3 (correlations between the parameters listed in columns 1 and 2) shows significant correlations between either the age of the subjects or length of submarine service and several of the biochemical or hematologic variables. Though there are many reports of age-related changes in the biomedical literature, there appears to be a consensus that corroborates most, but not all, of the significant correlations with age among the data from this study.

Total leucocyte counts for humans vary widely under normal circumstances and are altered greatly by many physiologic factors. Although distinct age changes in leucocyte counts have been documented for human subjects from birth to maturity, a relatively stable range during adulthood generally is expected (Miale 1972). No completely satisfactory interpretation of the available data has been found, however, to account for the age-related changes in white cell numbers recorded for the submariner population.

In an evaluation of the neutrophil data in Table 3, it must be remembered that these values are based on percentage, or relative counts, as shown in Table 1. The significant positive correlation of neutrophils with age, accompanied by a similar change for leucocytes, points to the possibility of an absolute increase in circulating neutrophils for these subjects as age increases. Indeed, calculations of absolute leucocycte numbers have verified the assumption that the age-related leucocyte changes are in large measure neutrophilic. The volatile nature of neutrophil responses to many frequently encountered circumstances such as hormonal changes, i.e., epinephrine, ACTH, emotional stress or even exercise—not to mention infections, other pathologies, or pharmacologic agents—adds to the difficulty of arriving at ultimate conclusions concerning the significance of the observed neutrophil changes at the present stage of these studies. The opposite trend in lymphocyte counts, a negative correlation with age, represents a relative change but not a statistically significant reduction in the absolute numbers of these cells with increasing age.

Hemoglobin and hematocrit values tend to decrease with age, in agreement with a broadly based survey of adults of the United States (Public Health Service publication, April 1967). The data presently available, however, do not show a significant correlation with age.

The significant decrease in the specific gravity of urine with increasing age may be postulated to result from either the changing fluid intake habits of the subjects or differences in the urine-concentrating ability of the kidneys as the population ages. The data indicate that the age-dependent specific gravity changes become statistically non-significant when the component arising from length of submarine service is removed.

The positive correlation of serum glucose levels with age seems to be well supported by the work of other investigators, particularly the finding of increasing concentration after a glucose loading test (Public Health Service publications, May 1964 and September 1966). In contrast,

considerable variability has been reported for the effects of aging on uric acid levels (Ludvigsen and Taylor 1970; Tolls, Werner, Hultin, and Mellecker 1970). In the case of this metabolite, an apparent increase in concentration with age in these subjects is reduced to a nonsignificant level when the small correlation with length of submarine service is removed.

The effects of age itself on serum cholesterol follow a pattern that would have been expected from previous findings (Department of Health, Education and Welfare publication, 1973). In males, cholesterol predictably increases with age during the age span of these subjects. The cholesterol concentration in these submariners, however, not only increases with age but also rises significantly with length of submarine service after correction for the influence of age. Because of the implication of serum cholesterol levels in the etiology of cerebrovascular damage (Department of Health, Education and Welfare publication, 1973), continued analysis of the relationship between environmental factors and living habits is warranted. Earlier reports of serum cholesterol changes during active submarine patrols lend support to the positive correlations reported here between submarine duty and cholesterol concentrations (Campbell and Rahe 1974; Shivertaker 1974). As a tentative hypothesis, it seems likely that the service-related tendency to accumulate increasing cholesterol levels may be associated with the semi-sedentary lifestyle imposed on these subjects by the confined submarine environment. The rich and generous diets served aboard submarines (Shivertaker 1974) may also promote long-term increases in serum lipids.

An additional observation that deserves specific attention is the effect of submarine service on glucose utilization after a loading test. Currently available data indicate an increasing rate of glucose utilization as a function of the length of submarine service. Such a situation might be of physiologic advantage if it were assumed that the decrease in glucose tolerance that normally accompanies aging, and which is demonstrated here, is detrimental to optimum health. On the other hand, an overproduction of insulin resulting in hypoglycemia after a carbohydrate load might be an early indication of a metabolic dysfunction associated with diabetes (Hoffman 1964). Again, both dietary habits and activity levels related to living aboard submarines may play significant roles in promoting metabolic changes, especially over a 20-year career in the submarine service. The ultimate effects on carbohydrate metabolism of the duty and habits associated with submarine life can only be determined by continued observation of these and other submariners.

The finding of a negative correlation between serum alkaline phosphatase levels and age seems consistent with other studies that report a sharp drop in the activity of these enzymes between adolescence and early maturity, and then a gradual rise commencing at the age of 40 to 50 years (Ludvigsen and Taylor 1970; Tolls et al. 1970; Fishman 1974). The significant age relationship with alkaline phosphatase activity may be somewhat exaggerated in these subjects because of the bimodal distribution of the population, in which age clusters occur at 19 to 25 and again at 30 to 36 years (Table 1).

A counterbalancing influence between the effects of age and submarine service, such as that apparently demonstrated for glucose tolerance, may also be seen for the serum alkaline phosphatase data. As noted above, the activity levels tend to decrease with age for this enzyme and increase with length of submarine service. Differential assays might be useful for determining whether the alkaline phosphatase from some particular tissue may be increasing in the serum with longer submarine service (Fishman 1974).

To examine further those data that seem to show significant correlations with either age or length of submarine duty in these men, split-sample correlation analyses were performed in which the population was divided in a pseudo-random manner into two subgroups, and the relevant correlation analyses performed for each of the subgroups. The resulting pairs of

correlation coefficients were then tested for their membership in a single population (Snedecor and Cochran 1967). These calculations demonstrated that the results shown in Table 3 are reliable, with the exception of the relationship between serum alkaline phosphatase activity and length of submarine service after correction for age (column R12.3, Table 3). The variability among the data obtained for this measurement may preclude the conclusion that time of submarine service is related significantly to this serum enzyme.

Though the findings of the present study add strong support to the observation that "the general health of the Polaris Navy must be considered outstanding" (Wilken 1969), it is apparent that considerable fundamental information concerning the relationship of biochemical and hematological responses to environmental, occupational, and aging factors may be obtained from a study of healthy subjects working in carefully controlled environments.

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